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7/25/68

THE EFFECTS OF MATERNAL STRESS
ON OFFSPRING BEHAVIOR

A THESIS

Presented to

The Faculty of the Graduate Division

by

Margaret Stephens

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Psychology

Georgia Institute of Technology

March, 1969

THE EFFECTS OF MATERNAL STRESS

ON OFFSPRING BEHAVIOR

Approved:

Chairman

Date approved by Chairman: 3-7-69

ACKNOWLEDGMENTS

The encouragement and support by the entire faculty and administrative personnel of the School of Psychology were instrumental in the production of this paper. Special gratitude is extended to members of the reading committee, Dr. E. Marks and Dr. K. Bachmann, for their careful evaluation of the study and helpful suggestions. Lastly, Dr. E. Jo Baker as thesis advisor has provided the ideal relationship between student and professor by encouraging self-reliance and critical awareness in her pupil.

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CHAPTER I

INTRODUCTION

The prenatal environment of the fetus is presently of concern to many fields of research. Numerous papers have been published on the effects of nutrition and the adverse action of drugs, chemicals, and x-irradiation on the developing fetus. Primary effects reported involve sensory impairment, structural deformities, and gross neurological changes such as microencephaly.

Since ethical considerations limit research on human subjects to descriptive studies, researchers have used animals in which these phenomena can be investigated through experimental induction of the desired environments. The most desirable experimental animal would be a mammal with a relatively short gestation period. The capability of producing sizable litters of offspring is also desirable to compensate for losses incurred as a result of experimental conditions. Rats satisfy these requirements; in addition they are practical experimental animals in terms of care and have more or less become the standard laboratory animal for psychological experiments. Albino rats were selected for this study due to the amount of literature in this field of research using them as subjects in this area. Of course, generalizations made about human behavior on the basis of results of this and similar studies should be viewed with caution.

To investigate changes in prenatal environment and their corresponding effects on the developing fetus, it was necessary to determine and control those variables that influence the measures and that could cause spurious results. A review of the literature (described in the next chapter) revealed many problems in experimental design and control of variables. In order to focus upon prenatal stress and postnatal maternal effects, variables other than these should be controlled. Among these control variables are genetic strain of mother and father, sex of offspring, genetic variation within a strain, the handling experience of the experimental groups, and age of offspring at testing. Solutions to some of these problems are discussed in the chapters on procedure and analysis.

One difficulty with research involving prenatal environmental effects is that investigators have not adequately specified how maternal stress was induced. For example, in the study of Joffe (1965) stress situation emotional response (crossing an electrified floor to obtain food and water) and a physical stress (electric shock) are confounded. Other studies have found that conditioned anxiety situations similar to those employed by Joffe lead to the development of gastric ulcers which are associated with emotional stress. Joffe's prenatal environment also included electric shock to the pregnant female.

The current research was designed to investigate the effects of one kind of maternal stress, physical stress, on offspring emotionality, activity level, and discrimination learning. These terms will be operationally defined in the chapter on procedure. Also of primary interest was the effect due to cross-fostering of the offspring, that is, what happens to a young rat whose biological mother was stressed while his foster was not, and vice versa.

Research hypotheses in this area can not yet be formulated on a theoretical basis. For statistical purposes the following null hypotheses were set forth:

1. There are no significant differences between groups as a result of their biological mother.

2. There are no significant differences between groups as a result of their foster mother.

3. There is no significant interaction between groups as a function of biological and foster mothers.

CHAPTER II

LITERATURE REVIEW

Previous findings on the effects of prenatal stress on offspring are reviewed here in chronological order. This research review has been restricted only to work done with rodents so that comparisons may be more easily made from one experimental condition to another.

W. D. Thompson and Sontag (1956) were among the first investigators to study the effects on offspring behavior of experimentally induced stress during the gestation period. The subjects consisted of mature virgin female albino rats, approximately 120 days of age. The animals were divided into two groups of six each. The control group received no special treatment during the gestation period, while in the experimental group, rats received two audiogenic seizures every day for 14 days. Upon birth of the offspring, litters of pups were exchanged across the two groups. The pups were weaned at 21 days of age and given the usual laboratory care until 80 days of age when testing began. The experimental conditions required that the rats learn to swim a 12 inch deep water maze. The criterion for learning the task was set at three successive errorless trials. Each animal was given one trial per day. Using the "t" test they found that the experimental group made significantly more errors than the control group (p   0.05), and required more trials to reach criterion. A general activity measure resulted in no statistical differences between the two groups.

In 1957, W. R. Thompson (1957) published a study dealing with the effects of prenatal maternal anxiety on emotionality in young rats. A strong anxiety reaction was induced in the animals by using a blocked avoidance technique. That is, prior to mating, experimental animals were given training in a shuttle-box. The rat was placed at one end of the apparatus. A buzzer sounded, followed by a strong electric shock. If the animal responded by running to the opposite end of the box before termination of the buzzer, it would avoid the electric shock. After mating, the pregnant experimental animals were placed in the shuttle-box. The buzzer was sounded; however, an obstruction was placed between the ends of the apparatus so that the rat could not run to the "safe" side. The avoidance response was experimentally blocked. No shock was given. The offspring of both groups were tested between 30 and 40 days of age in an open-field. The experimental animals were significantly more "emotional" as defined by the number of squares traversed in the open-field (inverse relationship with emotionality). A cross-fostering technique was employed; no postnatal differences due to foster mother were found.

Both of these studies report effects due to a prenatal stress treatment. But it should be pointed out that one factor was not controlled which may have actually produced these effects. In the W. D. Thompson and Sontag study, the control group was not subjected to any manipulation during gestation, so that a second variable may actually have produced the significant variation. This variable might be called the "handling" variable. Had the control group been subjected to the same manipulations as the experimental group with the exception of actually inducing seizures, that is, had the rats been placed in the same apparatus for an equivalent length of time but without auditory stimulation, then the author could have concluded that the seizures produced learning deficits. Because the control was not implemented, this conclusion should not be made. This has already been pointed out by Kaplan and W. R. Thompson (1957). Ader and Conklin (1963) investigated the problem of handling of pregnant rats. A procedure was employed whereby the manipulated animals were handled for ten minutes, three times daily throughout pregnancy. Offspring remained with their natural mothers or were cross-fostered within and between experimental and control groups. When tested at 45 and 100 days of age, the offspring of handled mothers were found to be generally less emotional than controls. Emotionality was defined in terms of open-field behavior and latency of emergence from a cage. Initially it may seem that the handling aspect would tend to decrease the influence of stress found in the Thompson studies so that stress actually would have been more effective than shown. In the Ader and Conklin study, handling was a gentling procedure acting to reduce emotionality while in the W. R. Thompson study, handling was a signal to the animal that stress was imminent, in this way acting to increase the emotional effects of stress. Because of this differential effect of the handling or gentling procedure, this variable is difficult to control.

Another stressor which has been relatively extensively studied is that of x-irradiation. The concern here is with detriments to the learning ability of the organism rather than to activity or measures of emotionality. Furchtgott, Echols, and Openshaw (1958), presented x-irradiation of 100, 200, or 300 r. to albino rats on days 14 to 15, 16, or 18 of the gestation period or immediately after birth. Beginning at the age of 45 to 50 days, subjects were given 25 trials in a Lashley III maze and retested following a 30 day rest period. Learning as measured in terms of trials to a criterion of two out of three errorless trials, total number of errors, and retention errors was adversely affected by irradiation. Between days 14 to 15, a 100 r. dose was effective, on day 18 a 200 r. dose, and neonatally only subjects receiving 300 r. showed learning decrements. Sharp, in 1961, expanded this kind of study to include measures of motor coordination of offspring. Nine pregnant rats distributed among three equal groups: a full-body irradiation group, a half-body group in which the lower half of the mother was shielded from the x-rays by lead, and a control group which received no radiation. The pups from these females were run in a 14-unit water T-maze 116 days after birth. Performance measures for the half-body group did not differ significantly from those for the controls. The full-body group differed from the other two groups in trials to criterion and rate of learning having higher scores on each. These conclusions led the author to suggest that the decrements in offspring learning were due to direct effects of radiation on the fetus and not to effects mediated through the mother. Wechkin, Elder, and Furchtgott (1961) included the variable of age in their study. The task studied was the albino rat's ability to climb an inclined plane. Subjects were irradiated on the 16th through 18th day of gestation with 200 r. and tested at the following ages: 4 to 5 months, 15 months, or 20 to 21 months. The experimental subjects were significantly inferior to controls and age was found to be inversely related to ability to climb an inclined plane.

Whereas radiation is a physical stress and has been shown to have effects on offspring behavior, an emotional stress, conditioned anxiety, shows somewhat conflicting results. Hockman (1961) was interested in testing the hypothesis that emotional stress undergone by rats during pregnancy can affect emotional behavior of offspring. At birth the offspring of experimental and control groups were cross-fostered. Open-field testing as the measure of emotionality was carried out on days 30 to 45 and 180 to 200. The overall activity in the open-field of experimental litters was significantly lower (indicating greater emotionality since emotionality is considered to be inversely related to activity in the open-field) on the early series of tests; however, the effect failed to appear in offspring raised by their own mothers. The interaction between prenatal treatment and postnatal separation experience was also significant. It is interesting to note that there was a high rate of mortality in the experimental group of mothers during the "stress" phase. Usually experimenters report many losses among experimental offspring but not their mothers. This suggests that the stress situations employed were indeed effective. Ader and Belfer (1962) published an article describing work similar to the Hockman study. It was found that, regardless of the sex and cross-fostering variables, the offspring of manipulated mothers were significantly less active (more emotional) than control offspring as measured by the open-field test at 30 to 40 days of age. None of the measures of emotionality yielded consistent differences between groups when tested at maturity. These results are in accord with previous findings and the authors interpreted them as supporting the contention that prenatal experiences are at least capable of influencing early subsequent behavior. However, it is important to note here that the measure of open-field activity was derived from a one-minute session in the field. The reliability of a measure during such a short time period might be questioned.

Morra (1965) published a series of studies which utilized tighter controls. He used conditioned maternal anxiety (blocked avoidance response) to induce differences in emotionality in young (25 days of age) rats. He also pointed out that the latter phase of pregnancy appeared to be more sensitive to these treatments. This result is in accord with the x-irradiation studies which isolated the 13th to 14th day of gestation as a critical point. The rates of viability and fertility were also affected by the prenatal treatments. The author could make no conclusive statements with regard to cross-fostering as a result of his design. He did not cross-foster because "... no primary effects of fostering were reported by other investigators." Although Thompson (1962) does not report significant main effects due to cross-fostering, he does report a statistically significant postnatal interaction effect with the experimental variable.

W. R. Thompson, Watson, and Charlesworth (1962) published a rather extensive paper covering some comprehensive research in this area. Briefly his results are as follows: offspring emotionality may be influenced by prenatal maternal anxiety with the effects diminishing at maturity; electric shock prior to pregnancy produced no significant differences in emotionality; the foster mother and biological mother had an interaction effect on offspring; adrenal injections decreased offspring emotionality and <u>increased</u> water-maze learning.

In 1963 Ottinger, Denenberg, and Stephens (1963) published a paper dealing with three experiments to test the hypotheses that offspring emotionality is positively associated with the mother's level of emotionality, as rated from open-field behavior when pregnant, and that offspring experiencing multiple mothering (mothers exchanged between two litters every 24 hours) are more emotional in adulthood than offspring reared by a single mother. Subjects were Purdue-Wistar rats. Offspring were tested in the open-field daily on days 50 to 53. Open-field emotionality data confirmed both hypotheses. The authors also established that offspring emotionality is independently related to both prenatal and postnatal emotionality of the mother. This study led to further investigations (Weir and DeFries, 1964; DeFries and Weir, 1964; Joffe, 1965; and DeFries, Weir, and Hegmann, 1967) into the problem of genetic factors, particularly interaction effects, between strains and prenatal treatment. It may be generally concluded that different strains of animals show different responses to similar treatment.

Finally, Joffe (1965) has published a study, the purpose of which was to determine whether subjecting female rats to a situation known to cause them physiological changes would have any effects on their offspring and, if so, to see whether or not the effects of conflict would be the same as the effects of other forms of prenatal maternal emotional arousal. The experimental animals were placed in a cage where the living area was separated from food (on one side) and water (on the other) by electrified grid flooring. For one hour in each 24, the current was terminated, allowing the animals free access to food and water. The control animals were subjected to the same living conditions and deprivation as the experimental group with the exception that no shock or conflict was employed. The females remained in the experimental cages for a total of 36 days, with a break of five days (during which they were mated) after 14 days in the cages. Offspring were tested at two ages, 78 to 80 days of age and 131 to 133 days of age. The first session was in an open-field and the second in the Hebb-Williams apparatus. This is a device for measuring the intelligence of rats as a function of how well they solve maze-like problems. No significant differences, other than sex differences, were found. On the Hebb-Williams apparatus, the offspring of experimental females made significantly more errors than the offspring of controls. The author accounted for his results, which did not agree with those of Thompson and Morra, by suggesting that the physiological changes in the study differed from those in other studies as a result of differing maternal stimulation, thus leading to different results. Another interesting aspect of Joffe's study was his decision to measure intelligence, as well as emotionality, in a non-stress situation (if food deprivation may be considered to be a non-stress situation). Previous learning tasks had employed the water-maze, a severely stressing apparatus for the rat.

CHAPTER III

INSTRUMENTATION AND EQUIPMENT

Maternal Stress Environments

The two maternal stress environments and a control environment were implemented as follows:

Electric Shock

Two Grason-Stadler rat test chambers, model E 3125A, with dimensions 9 1/4 inches x 7 5/8 inches x 11 1/2 inches, were wired in parallel to a Grason-Stadler Shock Generator, model E 10640S. The chamber floors consisted of 18 stainless steel shocking bars on 1/2 inch centers attached to multiple-contact connectors. Two opposite sides and the top were transparant plexiglas and the other two were insulated metal. The shock generator consisted of a shock source, electronic timer and grid scrambler. It delivered a 0.5 to 1.0 milliampere current to each chamber. A Scientific Prototype Timer, model 4052-J, timed both the stimulus and interstimulus intervals. All modules and control devices were mounted on a standard Georgia Tech Equipment Back. A simple relay system was wired to present the stimulus for five seconds with an interstimulus interval of ten seconds. The stimulus for this treatment group was electric shock. The power was supplied by a constant current generator, model 901, manufactured by Physiological Electronics, Inc.

White Noise

A speaker was placed adjacent to two chambers similar to those above. A Grason-Stadler noise generator, model 901B, delivered wide band white noise of approximately 75 decibels to the chambers through the speaker. It was similarly wired to the control panel and presented auditory stimulation at the same intervals as in the electric shock group. One switch activated both systems simultaneously.

Handling

Two metal cages of approximately the same dimensions as the test chambers, with two metal sides, two wire mesh sides and a grid floor were used to provide the environment for this group.

All chambers were placed inside an insulated container, two chambers in each of three such containers. These containers were three feet x three feet x three feet and prevented light from entering and noise from leaving the confined space.

Offspring Testing Apparatus

Open-Field

The open-field was a uniform piece of plywood, 35 inches x 35 inches x 1/2 inch, secured to a wooden frame. The top was painted flat gray and marked into 25 seven-inch squares.

Y-Maze

The y-maze consisted of a start box (stem of the y) from which the animal had two exit choices (each leg of the y). Each leg of the y contained the cues. The legs had one-way doors with plexiglas covers over the entire maze. The sides were approximately six inches high; the legs and start box were three inches wide. The floors of each leg were interchangeable; one was smooth and one had a textured surface. The entire maze was painted flat gray.

Activity Wheel

This piece of equipment was manufactured by the Grason-Stadler Company. It consisted of a cyclindrical wheel 20 inches in diameter into which the animal was placed. Running activity of the rats was measured by mechanically counting rotations of the wheel independent of direction of rotation.

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nant rats. For example, between 24 and 48 hours following birth, pups were coded and divided into three equal groups, each of which was placed with one of the three foster mothers. All foster mothers were given an equal number of pups to raise. The births of the offspring were staggered so that the nursing mothers were not given an overwhelming number of animals. A pilot study by the author indicated that this procedure is feasible. The animals were maintained on an ad lib feeding schedule and were disturbed as little as possible until weaning. The weaned offspring were then removed from the foster mothers and the intact groups were placed into group cages. Later all animals were placed into individual cages until the testing sessions were terminated.

Offspring Testing

Offspring testing was carried out in the same room in which the animals were caged. The living-testing room is similar in dimensions and other features to the maternal treatment room. All pups were tested between 90 and 100 days of age. The three measures taken were open-field behavior, activity wheel measure, and a discrimination task measure, in that order. Open-field and activity measures were made on the same day. Animals were then returned to their cages and their weight was reduced to 80 per cent normal body weight by a period of gradual deprivation. When they reached the 80 per cent level, about six days later, each rat was maintained at this percentage body weight until reaching criterion for the discrimination task. Deprivation provided the necessary motivation or incentive for learning. A correct response was followed by food reinforcement.

Emotionality Measure. For this study precedence was followed by defining emotionality as open-field behavior. It has been established that there is an inverse relationship between the number of squares which an animal will traverse in this apparatus and the animal's emotionality. That is, the more the animal explores the field the less emotional he is. This technique is widely used and is fairly reliable. The present writer prefers it because the animal does not interact, except in transportation and positioning in the field, with the experimenter. Such interactions (e.,g., King's Emotionality Rating) can lead to reactive measures of performance and may cause contaminations. Another measure which is made in the open-field is the number of boluses deposited. Defecation and urination are positively correlated with emotionality of the animal. Hall (1934) presents evidence that these are valid measures of emotionality. In the present study insufficient data were obtained from this measure since defecation in the open-field rarely occurred. Therefore, it was not used.

The animal was placed in the corner square of the field (same square for all animals). He was observed for a five-minute period; the number of squares traversed (or lines crossed with both forepaws) was recorded. The rat was then placed in the activity wheel. The field was cleaned following the testing of each animal to remove the odor from the previous animal.

Activity Measure. Each rat was tested in the cage of the activity wheel for a five-minute interval. The number of revolutions was recorded. This measure indicates the activity level of the animal. It is fairly reliable and has been extensively used in previous research. All tests were made between noon and 2:00 p.m.

In both of these measures, open-field and activity, only one time interval was used to obtain the animal's score for that test.

Discrimination Task. The last test made was a measure of the rat's discriminative ability in a tactual task. The y-maze was used in another study (Baker, 1968) and was found to be a moderately difficult task when used with tactile cues. In that research all rats reached one criterion level and the scores were approximately normal in distribution. Since this task had been effectively used in the present laboratory the decision was made to use it in the present research.

The floors, one smooth and one textured, were interchangeable and the number of right and left turns were counterbalanced. The animal was required to discriminate the grainy texture and run to that side (leg) of the maze. Alternation of cues between right and left legs was randomly made using a table of random numbers (Underwood, 1966). As previously mentioned motivation was provided by maintaining the animal at 80 per cent body weight. Reinforcement for making the correct response to the textured cue (floor) was two Noyes Precision food pellets, 4.8 millimeters by 4.9 millimeters, 97 milligrams.

In order to adapt the animal to the environment, that is provide him with "instructions" of what he is to do, he was allowed to explore the maze for four minutes with reinforcement on the textured side. During this exploration period the one-way doors were taped open allowing free access to each side. This procedure was used on the first day of the testing session. On the second testing day he was given four trials (with reinforcement and alternation of cues) with doors down so that he would experience nosing under them. On the third day he was given ten trials and continued on this schedule until he reached the criterion of ten successive errorless trials. This criterion was selected for learning since the probability of such a sequence happening by chance is quite low (p 0.01).

All testing sessions were appropriately counterbalanced across all groups for environmental conditions, such as time of day, and order of group tested. After completion of all three testing sessions the animals' code was read and sex was recorded. Animals were then placed on ad lib feeding schedules in the regular colony.

CHAPTER V

DISCUSSION OF RESULTS

Experimental Losses and Litter Sizes

Although these measures were not included in the proposal, as the experiment progressed a noticeable trend was evident; thus, the writer decided to analyze and report the following findings.

A standard Georgia Tech albino rat will produce a litter varying from eight to 16 pups with a mean of around 13 animals. The birth sizes for the nine animals in this experiment are given in Table 1. A one-way analysis of variance was performed on these scores (Ferguson, 1966) and a significant F ratio was found (see Table 2). These data confirm the results of Levine and King (1965) who found that treatment reduced litter size and increased infant mortality.

Table 1. Litter Sizes and Means for each Treatment Group

		Prenatal Treatment Group		
		E	W	H
Mother	1	6	10	16
	2	2	13	13
	3	5	10	14
mean		4.3	11.0	14.3
total		13	33	43

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There is little difference in the number of animals surviving with respect to each foster mother (15, 13, 11). The column factor (biological mother), however, is more complex. Since the biological mother kept her offspring for at least 24 hours, from birth until they were coded and cross-fostered, it is possible that she could have destroyed many of the young. Some animals have been known to consume their defective young leaving no evidence of their existence (Levine, 1965). Another factor was the significant difference among litter sizes which would also contribute to the different marginal column totals (5, 9, 25). In summary, each foster mother was given equal numbers of pups to raise and at weaning approximately equal numbers were present for each mother. However, each biological mother produced different litter sizes and may have destroyed numerous offspring before they were cross-fostered resulting in greatly disparate numbers at weaning. The next section will show how these losses affected the statistical design.

Statistical Design Paradigm

Due to losses among experimental animals before testing the frequencies were drastically reduced. This had serious consequencies on the proposed analysis. A split-plot design (Winer, 1962) was to have been employed so that within strain genetic variation would be taken into account. This would have refined the analysis by increasing its sensitivity to variation due to the treatment variables. The split-plot design required that offspring from each biological mother be represented in each subplot. Since some cells had frequencies as low as one animal, this requirement was not met. Therefore, a three by three complete factorial design was employed (Winer, 1962).

After compilation of the raw data the distributions were noted to be skewed. Homogeity of variance was tested by applying the F_{max} test (Winer, 1962). The smallest F_{max} observed ($F_{max} = 72.0$) was significant at beyond the .01 level. Since marked heterogeneity of variance and non-normal distributions (along with unequal cell frequencies) were present, the raw scores for each measure were transformed by taking their square roots. This transformation normalized the data and made a significant contribution toward homogeneity of variance. Cell and marginal frequencies appeared tied to the experimental treatments; therefore, a least squares estimation procedure was employed in testing the main and interaction effects (Winer, 1962). The individual analyses will now be discussed.

Analysis of Variance

A complete two-way factorial design was used to analyze separately the three behavioral indices. A two-factor paradigm was employed with three levels under each factor. (See Figure 1.) The error term used to test all hypotheses was the within-cell variance.

		Factor B: Biological Mother		
		E	W	H
Factor A: Foster Mother	E	Cell 1		
	W			
	H			Cell 9

Figure 1. Design Paradigm for the Analysis of Variance

Emotionality. Table 4 contains the cell and marginal means for the treatment groups. On the transformed emotionality measure (open-field traversals) no significant differences were found, $F_A = 1.80$, $F_B = 1.86$, $F_{AB} < 1$, $p > .05$.

Table 4. Means Computed on the Transformed Data for the Open-Field Measure

		B: Biological Mother			
		E	W	H	Marginal Means
A: Foster Mother	E	5.44	5.69	5.48	5.53
	W	5.57	5.27	4.52	4.77
	H	7.28	3.77	7.21	6.61
Marginal Means		5.83	5.13	5.69	5.58

This finding indicates that at maturity, offspring do not exhibit differences in emotionality as the result of prenatal maternal stress. Thompson, Watson, and Charlesworth (1962) concluded that there exists positive evidence favoring the hypothesis that prenatal stress can affect early offspring behavior; however, these effects appear to diminish with age. The effects reported also appeared to be largely independent of simple postnatal influences. It should be noted that these authors used conditioned anxiety to induce maternal stress, while the present author utilized a physical stress situation. Using maternal emotional arousal and physical stress, Joffe (1965) found no differences in emotionality.

Activity Level

An identical type of analysis was performed on the transformed activity scores. None of the calculated F ratios was significant; the only value greater than 1 was the interaction term ($F_{AB} = 2.07$). The present results do not indicate a significant difference in the activity levels among the three groups.

Discrimination Task

During the deprivation period two animals died and one refused to leave the start box in the maze. Removal of these animals reduced the total N to 36 for this analysis. The measure used was the number of trials required by the animal to reach the criterion of ten consecutive errorless trials. Means are given in Table 5.

Table 5. Means for the Transformed Trials to Criterion for the Discrimination Task

		B: Biological Mother			
		E	W	H	Marginal Means
A: Foster Mother	E	7.86	7.02	6.48	6.92
	W	7.14	7.38	7.11	7.18
	H	6.25	7.38	7.89	7.62
Marginal Means		7.39	7.25	7.14	7.21

The two-factor analysis of variance procedure as previously described was applied to the transformed scores. Two F ratios were significant, one main effect and the interaction effect. Table 6 shows the analysis of variance summary table. The significant main effect confirmed the alternate hypothesis that differences in discriminative behavior are, in part, a function of post-natal experience. Offspring reared by a mother (E) who had been shocked during pregnancy took fewer trials to learn the discrimination task than offspring reared by either W mothers or H mothers. This finding (main effect due to fostering) is in conflict with both the Joffe and the Thompson, et al., study. These researchers found cross-fostering effects only in interaction with other variables, notably prenatal variables.

Table 6. Analysis of Variance Summary Table for Transformed Measure of Trials to Criterion in the Discrimination Task

Source	df	SS	M.S.	F	p
A: Foster Mother	2	3.05	1.53	3.64	.05
B: Biological Mother	2	0.61	0.31		
A x B	4	6.20	1.55	3.69	.05
error	27	11.26	0.42		
Total	35				

The present study confirmed an interaction effect of postnatal treatment with prenatal treatment. ($F = 3.69$, $p. < 0.05$) There appear to be definite trends in the data. Foster mothers (E, W) which were stressed reared offspring which took fewer trials to reach criterion. The interaction between the treatment conditions (fostering and prenatal stress) indicates that the prenatal stress contributed to the observed differences among cell means. Figure 2 graphically shows this interaction. The W foster mother reared offspring which attained quite similar scores (7.14, 7.38, 7.11) showing very little difference as a result of prenatal stress. This indicates that the prenatal stress probably did not interact with the fostering variable. However, E and H foster mothers reared offspring which attained scores which varied as a function of their prenatal experience. It might be expected that the offspring which had not been stressed during the fetal period (H) would be the most proficient group especially in comparison with offspring shocked during the fetal period (E). This was true provided the E foster mother raised the pups. If the H foster mother reared the pups the offspring which were not stressed during the fetal period (H) performed the poorest. The E prenatal group functions in a reverse manner, i.e. offspring shocked during the gestation period performed better when reared by the H foster mother than by the E foster mother. The direction of these trends are intriguing and deserve further consideration in the form of more refined research techniques. Speculations concerning reasons for these results will be discussed later.

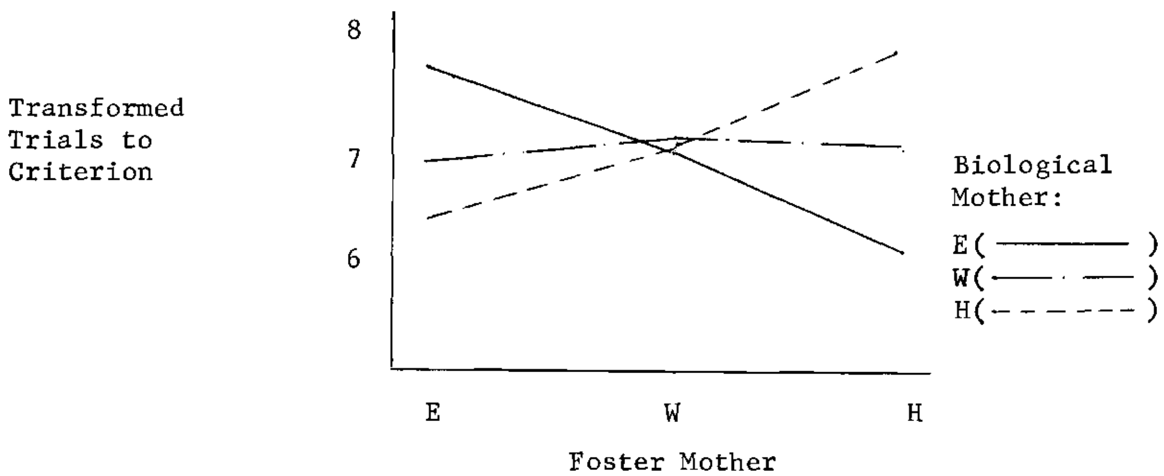

Figure 2. The Foster Mother by Biological Mother Interaction for Transformed Discrimination Scores

A final word should be said concerning the relationship between these results and prior research. Only three other studies have focused on learning indices and none of the tasks used (water mazes and problem-solving) were similar to the discriminative task used here. It is difficult to make comparisons, since the prenatal stress factor also differed across studies.

Correlation

Since a number of F ratios were being computed it was thought advisable to calculate correlation coefficients. The following Spearman rank order values were computed (Ferguson, 1966): activity level and open-field, 0.38; activity level and discrimination score, 0.06; and open-field and discrimination score, 0.34. None of these correlations was significant, which indicates that all measures were relatively independent in a probability sense.

CHAPTER VI

CONCLUSIONS

The results of this study support the data from previous studies which showed that prenatal maternal stress does not significantly influence the emotionality and activity of offspring at maturity (Joffe, 1965). The results of the discrimination task used in this study are unique in that no other research in this area has tested offspring on a discrimination task. Previous studies have used either a water maze (Thompson and Sontag, 1956; Sharp, 1961) or the Hebb-Williams Apparatus (Joffe, 1965). Both of the water maze studies reported significant prenatal effects. The present study does not. However, the prenatal stress (audiogenic seizures and x-irradiation) in each of the reported studies was extreme. The shock and noise levels used in the present study were well below the threshold required to induce seizures in the experimental animals. Cross-fostering was used as a control in each of the three previous studies; however, no significant primary effects due to postnatal experience (fostering) were found. The present study reports fostering effects as well as a significant interaction factor between biological and foster mother.

In comparing the present results with those found by Joffe, certain distinctions should be made between the different experimental conditions used by each author. Joffe used emotional arousal (as previously reviewed) in combination with a two to three milliampere electric shock.

This is a strong shock for a pregnant rat and may have induced seizures. However, he did report that the rats were not subjected to many seconds of this shock. The present study employed a 0.5 to 1.0 milliampere electric shock which is not much above that level which motivates or reinforces some behavior of the rat. That is, rats will bar press for mild electric shock in some situations. These differences in intensity of stimulation of the mother could account for the conflicting results.

Joffe suggested that "overstimulation" might have the effect of limiting the potential for neural modification and consequently decrease intelligence. Myelination which occurs too early, in effect, would reduce later problem-solving ability. The importance of prenatal stress is that the stimulation occurs during the embryonic period of formation of neural structures. Recent research into physiological mechanisms indicates that there may indeed be some neural changes taking place during gestation as a result of maternal stimulation (Levine and Alpert, 1959; Vernadakis, _et al._, 1967; and Petropoulos, _et al._, 1968). The important point is that such physiological changes might later affect the learning ability of the offspring. The present study does not entirely support such results. It could be that the Joffe study provided such intense stimulation that the neural structures instrumental in learning were detrimentally affected. The present study used stimulation of a far lesser intensity. It might then be suggested that while electric shock of 0.5 to 1.0 milliampere does not directly affect learning ability, it does indirectly affect the performance of the animal. If reared by the E foster mother, offspring ranked in performance as might be expected (stressed offspring being the lowest in performance) under the Joffe hypothesis of overstimulation. However, if reared by the H foster mother, the stressed offspring performed much better than non-stressed offspring. Although we have only considered the effects on the offspring of prenatal stimulation, it may be that certain changes have taken place in the mothers. Therefore, the present results could be accounted for partly in terms of the mothers' interactions with the offspring they are rearing. This is almost an obvious conclusion since the fostering main effect was significant. The stressed foster mothers reared offspring which performed better than the offspring reared by the non-stressed mother. Assuming differences among foster mothers as a result of their maternal experience (stress, non-stress), the following suggestions are set forth. The E foster mother was relatively severely stressed during pregnancy; therefore, she "rejects" or ignores the pups she is rearing. This differentially affects the pups depending upon their prenatal experience. The stressed offspring perform poorer on the discrimination task. The non-stressed offspring, perhaps because of increased sensitivity to environmental cues as a result of punishment or negative reinforcement by the mother, perform better. On the other hand, it may be that the stressed offspring have decreased their sensitivity to environmental cues as a result of interaction with the mother. The H foster mother was not stressed during pregnancy, and therefore, does not reject the offspring she is rearing. The stressed offspring that the H mother rears perform better than the non-stressed offspring on the discrimination task. Here, too, it may be argued that the sensitivity to environmental cues of either the stressed or non-stressed offspring changed as a result of interaction with the foster mother.

It is postulated that the stressed offspring do not utilize an extensive capacity to react to negative reinforcement or punishment administered by the foster mother (E), whereas the non-stressed offspring do use this capacity thus increasing their sensitivity to environmental cues which results in a higher performance level. A test of this hypothesis might be made by observing the animal's response to escape or avoidance conditioning. There is some evidence to suggest that some neonatal experiences lead to differential effects in these situations. It is also further suggested that stressed offspring retain an extensive capacity to react to positive reinforcement administered by the foster mother (H), whereas the non-stressed offspring do not utilize this interaction to as great a degree. Therefore, the stressed offspring obtain higher levels of performance under positive reinforcement. It follows, then, that the animal's capacity to learn a discrimination task somehow has been altered internally. However, the direction of these significant alterations is determined by the fostering experience of the animal and possibly by the reinforcement characteristics of the foster mother.

CHAPTER VII

RECOMMENDATIONS

The results of this study suggest many possible directions for future research. Further investigation of the physiological mechanisms which control these behavioral phenomena offers a fruitful area of study. The possibility that latent factors in the organism may function in specific reinforcement situations could contribute to directional trends in the data. The exact specification of the parameters which determine direction of effects is needed.

Parameters which already have been identified are prenatal variables, postnatal experiences, premating variables, strain or genotype, and condition of testing. Further research is needed to determine the differential effects of sex of offspring, such behavior phenomena as visual and auditory discrimination, espape and avoidance conditioning, and other performance measures. Of primary interest is the problem of degree of permanence of effects and the determination of shape of this function over time; that is, whether it is linear, exponential, etc. The foster mother's mediation of prenatal effects as well as specific changes in the biological mother during stimulation are interesting aspects of this area of research.

The primary and most important task for investigators in this area for the future is the organization of the experimental evidence into a coherent whole. The relationships between all parameters should be determined to provide coherence, and refinements within each variable are needed.

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